Polymeric Achiral Stationary Phases for the Analysis of Cannabinoid Mixtures

Dr. Weston J. Umstead, John M. Ferraro, Daicel Chiral Technologies

Introduction

The analysis of hemp-extracted mixtures has been a hot topic in the US since the passage of the 2018 Farm bill. The main focus initially was the quantification of tetrahydrocannabinol (THC) to ensure the cannabidiol (CBD)-containing products being produced remained below the stipulated 0.3% by mass set in the Bill.

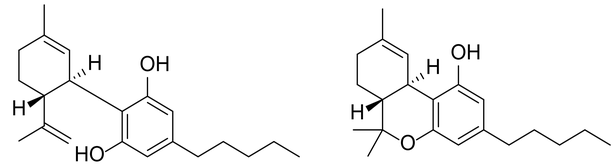

Figure 1:Cannabidiol (CBD; left) and Δ9 Tetrahydrocannabinol (D9 THC; right)

Since then, the focus has expanded to minor cannabinoid analysis, impurity isolation and analysis for quality reference standards, and even preparative scale production via chromatography[1]. The gold standard method conditions typically use an octadecylsilyl (ODS or C18) achiral column with a reversed phase mobile phase containing water and acetonitrile. For analytical applications, these methods are sufficient. However, the scalability of such methods for preparative applications are limited due to the need for large scale lyophilization and poor loadability of C18.

This study expands upon work previously published by the authors[2,3], by focusing on HPLC techniques for the analysis of a phytocannabinoid mixture, using polymeric achiral phases from Daicel Corporation. These novel phases were found to offer unique selectivity compared to currently available analysis method. Several column chemistries and mobile phase options are described, along with elution order determination and peak identification.

Experimental

The cannabinoid mixture and individual standards were purchased from Cayman Chemical Company (Ann Arbor, MI). The mixture contained Δ^8 THC, Δ^9 THC, cannabichromene (CBC), tetrahydrocannabivarin (THCV), cannabidiolic acid (CBDA), cannabigerol (CBG), tetrahydrocannabinolic acid-A (THCA-A), cannabinol (CBN), cannabidivarin (CBDV), cannabidiol (CBD), and cannabigerolic acid (CBGA).

The solvents used were all purchased from Pharmco and were HPLC-grade. The Hex was 95% n-hexane, and the EtOH was Reagent Alcohol (90% EtOH denatured with 5% Methanol (MeOH) and 5% 2-Propanol (IPA) v/v/v).

Chromatographic Conditions for the Separation of Phytocannabinoid Mixture		
Column	DCpak P4VP (250mm x 4.6mm i.d.), 5 µm	DCpak PMPC (250mm x 4.6mm i.d.) 5 µm
Mobile Phase	Hex-EtOH = 90-10	
Flow Rate	1.0 ml/min	
Detection	UV 230 nm ref. 450 nm	
Temperature	25°C	
Sample	Mix – 100 µl of stock solution evaporated and re-dissolved in 1 ml of 9:1 = Hex-EtOH Single Compounds – 1 mg/ml in Hex-EtOH = 9:1	
Injection Vol.	10 µl for mix; 5 µl for individual cannabinoids	

The HPLC used in the screening and optimization was an Agilent 1200 configured with low-pressure mixing, quaternary mobile phase delivery system, vacuum degasser, autosampler, and photodiode array UV detector. Column temperature was not controlled for screening, but was held in a column oven at 25°C for optimization. The instrument was controlled by Agilent ChemStation RevB.04.03[16].

Results and Discussion

The cannabinoid mixture was screened on 2 achiral columns using a mobile phase of Hex-EtOH = 9:1 and Hex-IPA = 9:1, as it was found these mobile phase ratios afforded good retention and selectivity. Generally speaking the separations with IPA didn't produce good peak shape relative to the EtOH separations. Peaks were broader and didn't yield as good of a selectivity.

Both DCpak P4VP and PMPC afforded resolution of most peaks, with PMPC giving a shorter retention time compared to P4VP (Figure 2).

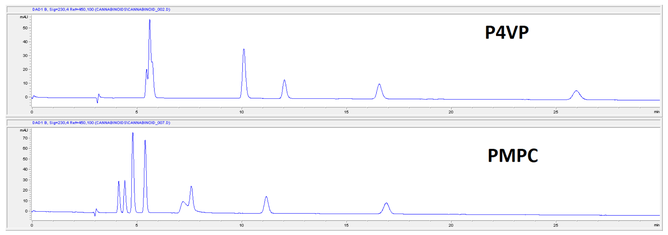

Figure 2: DCpak P4VP and PMPC with phytocannabinoid mixture using Hex-EtOH = 90-10

No optimization from the screening was required, so individual standards for the mixture were prepared and run to determine the elution order. The elution order for P4VP is shown in Figure 3.

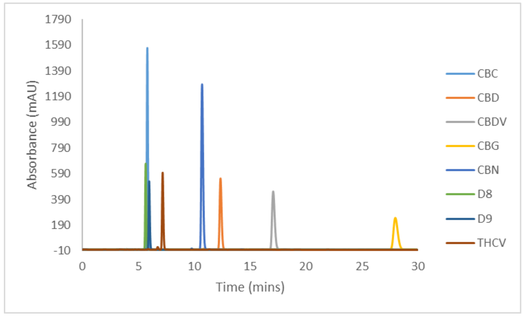

Figure 3: Cannabinoid elution order on P4VP

There are several co-elutions at the beginning of the method from D8 and D9 THC with CBC. Future work will be aimed at improving the resolution. A partial elution order was performed on PMPC (Figure 4). Both methods show excellent resolution between THC and CBD, which still remains one of the priority separations for hemp extract release testing

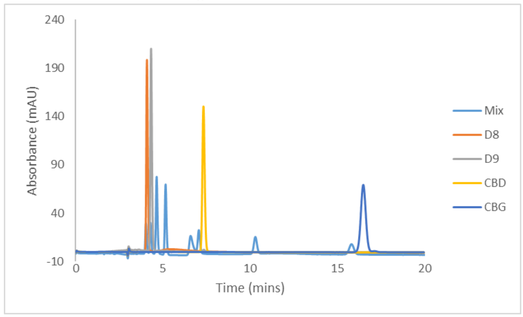

Figure 4: Abbreviated cannabinoid elution order on PMPC

Conclusions

Daicel's polymeric achiral columns were found to give very good resolution of the components of the phytocannabinoid mixture from Cayman. In particular, P4VP and PMPC both provided sufficient separation of THC from the other mixture components. Future work for these columns and methods include testing on real hemp extract samples and determining potential preparative productivity.

References

1. Ferraro JM, Umstead WJ. Chiral Separation of Cannabichromene, Cannabicyclol, and Their Acidic Analogs on Polysaccharide Chiral Stationary Phases. Molecules. 2023 Jan 24;28(3):1164. doi: 10.3390/molecules28031164.
2. Denicola, C.; Barendt, J.M. Cannabinoid Isolation Models Utilizing Immobilized Chiral Stationary Phases and SFC. Cannabis. 2018.
3. Umstead, W., Separation of the Enantiomers of (±) Δ8-THC and (±) Δ9-THC. In Application Note; Daicel Chiral Technologies, West Chester, PA, USA: 2019.

DAICEL Chiral Technologies

Web: www.chiraltech.com
Email: chiral@cti.daicel.com
Phone: 610-594-2100
Fax: 610-594-2325